AMERICAN JOURNAL OF BOTANY

Vol. III | July, 1916 | No. 7

THE DEVELOPMENT OF THE PHYLLOXERA VASTATRIX LEAF GALL

Harry R. Rosen

I. Introduction

Phylloxera vastatrix Planchon [Stebbins (27) uses the synonym *Phylloxera vitifoliae* Fitch] is the plant louse which was introduced into Europe from America and became one of the most dreaded enemies of the European grape vine. On American vines the insect usually attacks the leaf producing ugly cecidial growths but not seriously impairing the health of the plants attacked. On European vines, however, the insect does most of its work on the roots, becoming a very destructive pest. Much has been written concerning the life history of this parasite, a large part of which has been brought together by Viala (29).

II. Occurrence and Appearance of the Mature Gall

The material for this paper was gathered in the vicinity of Madison, Wis. The profuse growth of the wild vines of *Vitis vulpina* L., together with the ease with which one can find the Phylloxera galls, made this gall a desirable one to study in this region. My observations extended over a period of two growing seasons, during which time I have been able to observe hundreds of these cecidia. Although Cook (6 and 7) and Stebbins (27) found the galls on both the upper and lower surfaces of the leaves, I have found them only on the lower surface. Houard (15) and Cornu (8) mention only the lower surface as the bearer for the

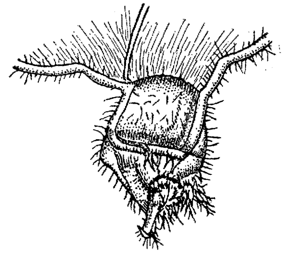


Fig. 1. A gall as seen on the lower surface of the leaf through a hand lens. Magnified about 3 times.

[The **Journal** for June (3: 261–336) was issued July 15, 1916.]

European galls. Very frequently the lower surface would be completely spotted with these wart-like outgrowths. A good-sized gall is about as large as a pea. It appears, on close examination, as a much furrowed and wrinkled, irregular pouch, with hairy projections, the mouth of the pouch opening on the upper surface of the leaf. When the gall has reached maturity the mouth is marked by two lip-like growths extending 2 to 3 mm. above the upper surface of the leaf, while around these lips a profuse growth of glistening, downy hairs covers and entirely closes the opening to the cecidial cavity.

III. Histology of the Normal Leaf

It is very evident that, in order to understand fully the changes which occur in the *Vitis vulpina* leaf during gall formation, it is necessary to have a good understanding of the structure of the young and of the mature normal leaf. Cook (4) gives a drawing of a normal leaf of *Vitis vulpina* and notes that, as compared with other leaves, the palisade is not pronounced, while the mesophyll is more compact. Comparing gall structure with the structure of the normal leaf, he

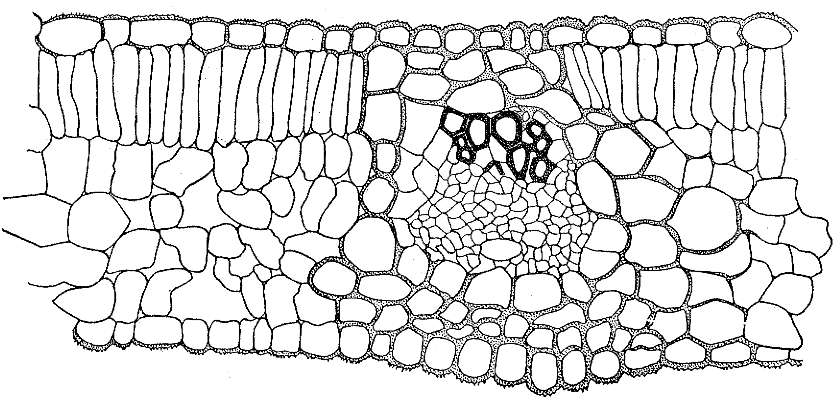


Fig. 2. A cross section of a normal leaf, magnified about 349 times.

points out that it is necessary to compare the gall structure with the structure of the leaf on which the gall is found, and not with the typical leaf. I have found, contrary to Cook, a well-developed palisade, and a spongy mesophyll with numerous air spaces in mature, gall-bearing leaves. Text-figure 2 shows a drawing of a cross section of a mature leaf taken from a portion immediately adjoining a fully

developed gall. Cook's figure leads me to suspect that he did not have a fully developed, normal leaf. The figure which he gives of the gall would seem to substantiate this view, since this figure does not appear to represent a typical fully developed gall as Cornu (8) or as I have found it. However, since gall formation always begins on the very young, embryonic bud leaf, it becomes necessary first to consider the structure of this leaf. Text-figure 3 shows a drawing of a cross section of such a young leaf. The diameter of this leaf is about one-half that of the fully developed one; the average diameter of the mature leaf is about 144 μ and of the young leaf, about 68 μ. A close examination of the young leaf figured below shows two epidermal layers with as yet no development of cuticle, an abundance of rather

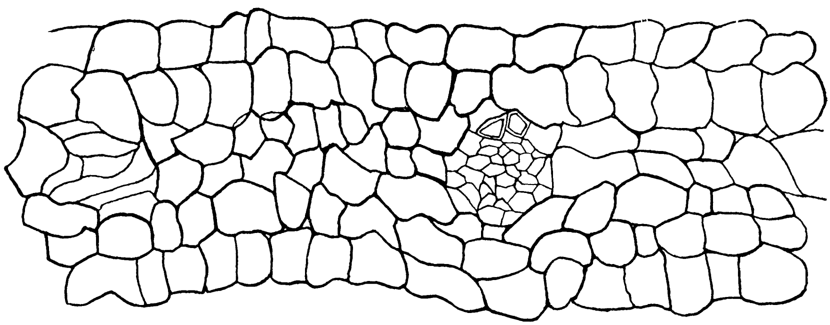


FIG. 3. A cross section of an embryonic bud leaf, magnified 574 times.

rigid, unicellular hairs around the margin of the leaf and on the larger veins, with a few very sparsely scattered hairs over the lower and upper leaf surfaces, an embryonic palisade layer made up of cells not much longer than they are wide and showing very little variation in length as compared with their width, and an embryonic spongy mesophyll made up of 3 layers of cells, so close together that scarcely any air spaces can be observed.

IV. Variations in the Appearance of the Gall

Here it should be noted that, although superficially the galls appear very similar, a closer examination reveals many variations in the general size, form, number of hairs, and in convolutions. These variations are mainly due to the locality on the leaf selected by the insect, as a gall started on a primary vein is quite different from one

started between the veins. A primary vein in a very young leaf is made up of two epidermal layers with a large number of unicellular and a few multicellular hairs, which arise from both layers, a mass of parenchyma cells which make up a large part of the vein, surrounding the vascular elements proper and later on becoming thick-walled supporting cells; the xylem consists of several vessels usually having close and loosely woven spiral thickenings, while the phloem consists of a few (about 30), elongated sieve cells. One of the striking features with reference to the arrangement of galls on the leaf is the fact that they so frequently occur along the veins, especially along the largest veins. The mouth of the gall, which is the hairy opening on the upper surface of the leaf, usually is not circular but has a longer diameter parallel to the axis of the vein. This seems to be due to the fact that the insect places herself with her longitudinal axis parallel to that of the vein. Figure 2 shows the insect in this position, although she is

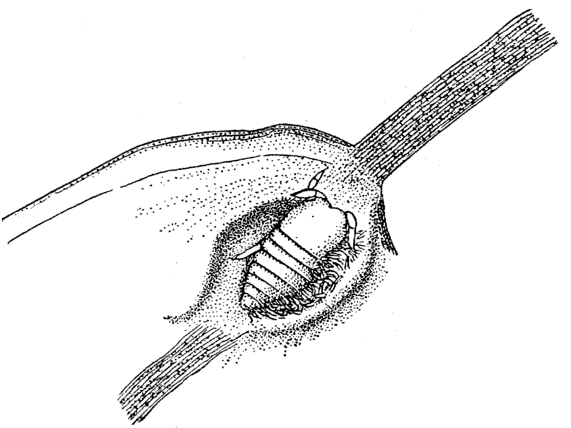


FIG. 4. A nymph attacking a primary vein, resting in the depression.

partly contracted away from the surface. Text-figure 4, however, leaves no doubt concerning her orientation, the insect being shown with her longitudinal axis parallel to the direction of the primary vein, which she is vigorously attacking.

The growth of the gall keeps pace with that of the young leaf. Should anything retard the growth of the leaf, that of the developing gall is correspondingly inhibited. Likewise, should anything happen to the insect, the gall ceases to develop. This has been observed by

Riley (24) and by Cornu (8). Successive daily measurements of the growth of galls shows that 12 to 15 days are necessary for complete development.

V. Histological Development of the Gall

During the winter of 1913, cuttings from a *Vitis vulpina* vine were grown in the greenhouse, and in the following spring, when the first galls appeared out of doors, some of the nymphs were taken out of a gall and placed on young leaves of cuttings growing in the greenhouse. Twenty-four to forty-eight hours later, the first signs of gall formation appeared. The nymph, which had been placed on a bud leaf, as the bud was opening, had located itself on a primary vein, and as the young leaf grew free from the bud scales and opened so that its whole upper surface became visible, the insect was seen in a shallow depression in which it was resting. This marks the first outward sign of gall formation. On the upper surface of the young leaf the gall appears as a shallow depression measuring about 0.5 mm., in depth and in width, the margin of which shows a growth of fine upright hairs, while the under surface of the gall shows a corresponding convexity as compared with the rest of the lower surface.

Text-figure 4 shows an early stage of gall formation such as described above. The figure was drawn under a binocular microscope while the leaf and insect were still living, and shows the insect at work on a vein, lying in the depression and surrounded by the hairs, both of which are the results of her labor. Ráthay (23) gives good colored drawings of the outside appearance of these early stages of gall formation.

In order to follow the histological development of the gall, different stages were collected, fixed in various dilutions of Flemming's, Carnoy's and other fixatives, imbedded in paraffin and subjected to various stains. Nearly all material was cut so as to give a serial arrangement of sections.

The first sign of gall formation in 1914 did not appear until late May. Opening buds from any region of a vine showing galls on the expanded leaves were brought into the laboratory and examined for signs of the grape-vine Phylloxera. Fortunately, I was able to find very early stages in great abundance. Figure 1 shows a cross section of a gall which is not more than twenty-four hours old; the hairs

of the upper and lower epidermis of the leaf adjoining the midrib are the results of the first twenty-four hours of insect attack.

Concerning the histological structure and development of the gall, brief descriptions are given by Cook (4), Pantanelli (22), and Cornu (8 and 9). As already pointed out, gall formation starts on the young bud leaf, where the insect usually places herself along a primary vein, either directly on top of the vein or directly alongside of it, and an abnormally large number of unicellular and multicellular hairs, grow up around her from the upper epidermis. The lower epidermis likewise feels the stimulus and the abnormal production of similar hairs is started. The tissue beneath the insect appears sunken so that a basin-like cavity is produced on the upper surface of the leaf, in which the insect lies. Figure 2 shows the insect drawn away from the hollow in which it was resting, but a portion of its proboscis is still seen piercing the tissue of the vein.

The apparent sinking in of the upper tissues of the leaf is due to a partial collapse of the young mesophyll tissue. Figure 9 shows a cross section of a young bud leaf. The proboscis of the insect is seen piercing through the epidermal cells, and appears broken off from the rest of the insect, which is drawn away from the leaf. The proboscis, curved around and reaching to the phloem of the vein, was followed in three successive sections, only one of which is shown. The narrowness of the leaf where the setae of the insect are projecting, and the papillate projections bordering the hollow are worthy of note. The constricted portion measures 72μ as compared to the normal unconstricted portion, which measures $96\ \mu$. Comparison of the cells in the two regions shows that this difference in size between the constricted portion and the unconstricted portion is due to the difference in size of the mesophyll which measures $40\ \mu$ at the constricted part, where the proboscis protrudes, as compared with the normal unconstricted mesophyll which measures 68 microns. Thus we see that the first twenty-four hours of insect attack brings forth a decrease in size of the portion attacked, accompanied by the production of hairs around the constricted portion, which is a hypertrophic effect. Reasons for these developments will be discussed later.

Following the histological development of the gall, we find after three to four days of insect attack, that rapid proliferation of the underside of the leaf begins to take place, while the tissue of the upper side shows a slight enlargement of the palisade cells and of the upper

epidermis, perpendicular to the surface of the leaf. Figure 3 and higher magnifications of the central part of the same section in figure 4 show the rapid proliferation of the mesophyll of the lower half of the leaf, together with slightly enlarged palisade cells, which only show this enlargement as they distance the proboscis. These two figures are especially noteworthy since they show the proboscis seemingly at work, and extending through the whole width of the leaf. So far as I know, they are the only figures of their kind which have yet been published. The insect is shown resting in a rather deep cavity instead of the shallow depression as it did after twenty-four hours of attack. During this three to four days of insect attack the young bud leaf has had an opportunity to unfold, being two to four times the size which it had at the time the insect first started its attack. It is necessary to bear in mind that the pouch-like form of the gall, which is beginning to manifest itself at this age, not only represents an excessive local growth, but also represents a change in the direction of growth of the tissue attacked. Instead of taking the normal direction of growth, the portion of the leaf beneath the insect grows downward and takes the form of a pouch. This growth downwards, is accentuated by the fact that the lower half of the leaf which does most of the proliferating, grows downward, in the path of least resistance, while the upper half enlarges slightly, without cell divisions.

It is a peculiar fact that the direction of growth in this gall, as in all other galls where the gall producer is situated at one side of a plant organ, is always away from the insect, opposite to the direction of the application of the stimulus resulting in a sort of negative tropism. Küster (12) says: "The side growing most in a leaf gall is always the one away from the gall animal, so that in this rolling of the infected leaf area, the gall animal comes to lie within the cavity thus produced." However, in this gall, although most of the hyperplastic tissue making up the gall comes from the lower half of the leaf, lying beneath the insect, the upper half of the leaf, which goes to make up the sides of the cavity, grows extensively upward. Pantanelli (22) describes this proliferation of the upper epidermis and palisade cells and points to the fact that growth due to the excessive proliferation of these cells finally forms the "lips" of the gall and almost completely encloses the insect.

The striking histological peculiarity of figures 3 and 4, representing 3 to 4 days of insect attack, is not the excessive growths of the lower

half of the leaf, since such growths have been described for other galls, but the *lack* of proliferation of both the upper and lower halves of the leaf in the portion immediately around the proboscis, which makes this part appear as a narrow neck between two masses of hyperplastic growths. This is a development which has not yet been described for other galls.

Other features which are worthy of note, at this stage of gall development are represented in figure 7. This figure, which is a highly magnified view compared with the other plate figures, shows a cross section of gall tissue taken from the portion below the insect. Starting from the top, which lined the base of the insect cavity, we see three layers of elongated cells, representing the upper epidermis, the palisade layer, and a layer of irregular mesophyll. As compared with corresponding normal leaf tissue, these cells show marked hypertrophic growth. The walls of these elongated cells, especially the walls running parallel to the leaf surface, are very much thickened in some places and entirely lacking in other places of each individual cell. In some cells, all that is left of the walls are narrow threads, which often show a reticulate arrangement and stain red with safranin. Such cell-wall features are as rare in galls as in any other pathological structures. Weidel (31) reports a dissolution of walls in the larval chambers of Cynipid galls and P. Magnus (19) finds a sieve-like dissolution of the cell walls produced by a Urophlyctis, which he figures. This latter case is more comparable to figure 7 since Weidel reports dissolution of whole walls in his insect galls. It seems that the cells shown in this figure might possibly assume the characters of tracheal elements, similar to that found by Küster (12) in leaf callus of Cattleya. In the outer cells of this callus, he found reticulate thickenings, which in the lower part of the cell are only narrow meshes between single thickened bands, while in the upper part the bands are usually flatter and somctimes partially interrupted. As translated by Dorrance (12), he says: "This case is of special interest since, aside from tyloses, it is the only one known to me in which hypertrophic growth, incited by a wound stimulus, is combined with the formation of a special kind of wall thickening." In the Phylloxera gall we likewise have a thickening and a partial dissolution of hypertrophied cells, giving the appearance of tracheal formations as seen in wound callus of leaves, described by Küster. This reticulate structure is only seen in younger galls, due to the fact that as the gall reaches maturity the cells at the

base of the insect cavity collapse and their individuality is lost. These are the most noteworthy histological developments seen at the end of three to four days of insect attack.

At the end of five to six days of cecidial development, the cells of the palisade layer at the base of the insect cavity can be distinguished from the rest of the mesophyll, while at the sides of the cavity their identity is lost and they assume the characters of rapidly dividing thin-walled parenchyma cells, sometimes enlarging enormously and becoming isodiametric or irregularly ovoid in shape. The rest of the mesophyll below the bottom of the cavity, except in the region of the proboscis, continues to proliferate enormously into a mass of thin-walled cells. As to the epidermal cells, some of them enlarge in the plane perpendicular to the surface of the leaf, as was shown in figure 7 for the earlier gall development, but at the upper part of the cavity they give rise to a large number of multicellular hairs. The lower epidermis likewise produces many such hairs.

The daily growth in size of the gall is very marked. In twenty-four hours it may increase 0.3 mm., in its long axis, so that proliferation and enlargement is very rapid. At the end of twelve to fifteen days, when the gall reaches maturity and the normal leaf has attained its maximum size, a section through the side of the gall, cut perpendicularly to the leaf surface, reveals an enormous mass of thin-walled, partly empty parenchyma cells, some of which are greatly elongated. This is the most striking feature in the mature gall.

The histological structure of the mature gall is as follows: Starting with the lower epidermis, we note the small number of stomata, while the epidermal cells show very little cuticular development. The epidermal cells are usually smaller or the same in size as the normal epidermal cells, although they sometimes appear elongated and narrow, running parallel to the surface of the gall. The corrugations or striations of the cutinized layer of the normal leaf are absent in the epidermal cells of the gall, except where it bounds vein parenchyma, in which case the striations are very evident. The mesophyll is made up of a mass of cells, many of which are rather undersized, as compared with normal mesophyll cells, while others are usually elongated perpendicularly to the surface of the gall as noted by Cook (4). However, they sometimes turn sharply and run parallel to the surface of the gall. This condition is brought out in figure 5. The arrow is pointing to groups of such elongated cells which are bent at right angles to the

surface of the gall. But the striking feature in this huge mass of tissue is its compactness and the total absence of air spaces. As Cook points out, the palisade cells cannot be distinguished; at the bottom of the cavity they are found in a more or less collapsed condition while at the sides they are usually not to be distinguished from those of the mesophyll. The vascular elements have also been changed from their normal arrangement. Although in total amount they are not noticeably increased, they are frequently scattered and twisted into separate small groups. Figure 6 shows how the xylem and phloem from one vein are separated and scattered by wedges of parenchyma cells, which usually show thickened walls. Compare this scattered mass of vein tissue in the center of the gall with a normal vein, marked N, in the same figure. Sometimes the vascular elements in the gall take the form of cylindrical or circular masses of cells which reminds one of the ball-like groups of tracheids that Küster (12) describes in callus tissue. What has been said for the lower epidermis can also be said for the upper which lines the cavity of the gall; besides that, at the base of the cavity the epidermal cells as well as several layers of mesophyll are totally or partially collapsed.

VI. Chemical Constituents of the Phylloxera Gall

Figure 8 is a photomicrograph of a section cut from the center of a mature gall. It represents a very low magnification as compared with the other plate figures. Some of the eggs and nymphs are still visible inside the cavity of the gall. This section also gives a good view of the so-called "nutritive zone" of the gall, described by Cook (4). It is represented by the very dark mass of cells below the cavity of the gall. These cells are filled with tannin, crystals of various forms, starch grains and other substances; because of this, this portion of the gall stains heavily. Pantanelli (22) has made a chemical analysis of this Phylloxera leaf gall and finds that gall-bearing leaves contain more total organic nitrogen and more proteic nitrogen than normal leaves; the ash content is lower in lime, iron and magnesium in the gall-bearing leaves. He finds an abundance of starch, albumin, fat and phosphates in the nutritive zone.

Molliard (20) made a chemical analysis of two leaf galls of the elm, both produced by plant lice, *Schizoneura lanuginosa* Hartig and *Tetraneura ulmi* De Geer. Comparing the same weight of gall tissue with

normal leaf tissue he finds that galls contain a greater percentage of water, a smaller percentage of ash, a total absence of sucrose, a greater percentage of reducing sugars, four times as much tannin, a smaller total nitrogen content but a greater percentage of soluble nitrogen. His results are quite similar to those obtained by Pantanelli with the exception of the total nitrogen content. Molliard thus points out that his results as well as those of Pantanelli, and Paris and Trotter, indicate an increase in simple substances in galls. Furthermore Molliard finds an enhancement of respiration, an increase in the oxidases laccase and tyrosinase, and in free acid. He concludes that the insects inject into the plant tissue certain enzymes, which may explain the presence of a large amount of simple substances.

VII. Discussion of the Stimuli Producing Galls

In stimulated structures generally, we may find an increase in enzymes, as Czapek (11) demonstrated for geotropically stimulated roots, where he found an increase in oxidases, especially tyrosinase. Von Schrenk (30) likewise found an increase in oxidizing enzymes in intumescences produced by sprays of various copper salts on cauliflower leaves. It seems, therefore, that the presence of a large amount of these enzymes in galls, especially in the Phylloxera gall, does not necessarily mean that they have been injected into the attacked tissue, but on the contrary, from our knowledge of enzymes in other stimulated structures, there seems to be good reason to assume that such is not the case.

Before going further into a discussion of stimuli producing galls, it will be best to discuss the early development of the Phylloxera gall. We noted in figure 9, which represents gall formation at the end of the first twenty-four hours of insect attack, that the portion of the leaf beneath the insect, in the vicinity of the proboscis, is constricted and measures 72 μ as compared to the normal width of the leaf, which measures 96 μ. Furthermore, it was shown that this difference in size between the constricted and the unconstricted, normal portion, was due to the decrease in size of the mesophyll of the attacked part. Besides this, we noted the beginning of abnormal hair production at the borders of the depression.

How has the insect brought about the decrease in size of the attacked mesophyll and the consequent depression? Is it due to

sucking out the contents and a consequent decrease in size of the portion attacked? This seems the most reasonable explanation. Although a few of the upper epidermal and mesophyll cells are killed (I have never found more than two or three epidermal and two mesophyll cells killed), by the action of the proboscis, the hollow is not the result of their death and a subsequent sinking in of the neighboring tissue.

Employing the technique used by Barber (2 and 3), a considerable number of fine, capillary glass tubes were made measuring from 5 to 20 μ in diameter and around 0.5 mm., in length. About 25 of these tubes were stuck into very young vine leaves and allowed to remain there until the leaves had grown to a fair size. Several punctures in small leaf areas were made in some cases and a circle of india ink was drawn around each area to indicate the place of operation. Young leaves punctured in this manner were also permitted to grow to a fair size. Sections were then made of the wounded leaf areas, and while microscopic examination showed dead cells in the punctured regions, no depressions were found around the point of injury. These experiments add weight to the belief that the depression in the vine leaf below the insect is not due simply to a puncturing by the insect's proboscis. Furthermore, in figure 9, it is seen that there is a depression not only of the upper leaf tissue but also of the lower, where the proboscis has not gone through and has not killed any of the lower epidermal cells.

It occurred to the writer that the force exerted by the insect's body pushing against, and weighing down upon a delicate, embryonic leaf, might have something to do with the formation of the depression. The furrows made by twiners on growing plants would perhaps be an example of the effect of such a force. Moreover Molliard (21) has noted the fact that a pressure exerted upon the surface of a growing portion of a plant may cause a depression at the point of contact, and an increase in growth, a hyperplasia, in portions adjoining the depression.

To test the effect of a force comparable to that exerted by the body of the insect, fine glass needle points, made in the same manner as those described above, measuring 5 to 20 microns in thickness and several millimeters in length, were held over a very small flame, so that the heated end coiled up and consolidated into a small glass knob. The tubes thus treated appeared as very small, round-headed,

glass pins. These were so stuck into the young vine leaves that the heads pressed against the surface of the leaves. The pressure of the pin head was to take the place of that of the insect body. After the leaves had grown to a considerable size, microscopic examination of the injured areas showed dead cells which the pins had pierced. A slight depression was noticeable where the head of the glass pin had pressed against the leaf surface, but nothing was obtained resembling the insect cavity, with its fringe of hairs.

It seems, therefore, that, at the beginning of its work, the insect by its sucking has caused a partial collapse of the tissue attacked, or at least a cessation of its growth, resulting in a sunken area or hollow in which the insect rests. What is the cause of the enlargement of the epidermal cells and why do they divide to produce multicellular hairs?

The production of hairs occurs quite commonly in gall formation. Erineum galls are almost entirely given over to the formation of hairs. As to the abnormal production of hairs other than in gall formation, Haberlandt (13) caused groups of colorless hairs to be formed by destroying transient glandular hairs of *Conocephalus ovatus* and *C. suaveolens*. The glandular hairs functioned in eliminating water and their removal, according to Haberlandt, resulting in a surplus water supply, caused the formation of intumescences consisting of bunches of hairs. Küster (12) points out the similarity between epidermal leaf hairs of Erineum galls and root hairs. He cites Schwartz as stating that changes in cell turgor may cause abnormally large root hairs. Sorauer (26) noted that wooly tufts were produced on the inner side of the core of the apple, which he assumes were due to an excess of water. This formation, Küster says, resembles callus tissues.

We note that in these cases a pressure stimulus has been put forth by the various investigators, as the initial factor in the abnormal production of hairs. The removal of certain organs or a retardation of a function of certain parts induces an abnormal pressure on adjoining parts which results in increased growth.

As stated above and shown in figure 9, the insect causes a decrease in size of the mesophyll cells in the region where the proboscis is at work, a decrease which seems to be due to a retardation in growth, and which makes this part appear as a depression in the leaf. Around the periphery of this depression thus caused, hairs are formed. May it therefore not be assumed that these hairs are the result of the

stimuli caused by change in tension and pressure brought about by the partial collapse of the attacked mesophyll? The evidence presented above by various investigators seems to substantiate such an explanation to account for the abnormal production of hairs.

Küster claims that in Erineum galls the stimulus causing the abnormal growth of the epidermal cells "comes from a poison which the gall insects produce, concerning which nothing more is known." This theory, as well as any other *chemical* theory, makes it difficult to explain the production of hairs in the Phylloxera gall. If we assume that a chemical substance is introduced into the leaf by the proboscis, then we must also assume that this substance, starting from the proboscis as a center, should diffuse or osmose equally in all directions; or, if on the other hand we assume that the substance given off by the insect is not readily diffusible in the tissues but that it initiates certain stimuli, which can be felt some distance from the initiating center, then we must also assume that changes or responses induced by these stimuli should appear equally distributed in all directions. But in the Phylloxera gall, as I have pointed out, the response is rather unequal, none or few hairs are produced in the depression, but they are produced always with perfect regularity at the edge of the depression. Does not the character of the response seem to point to a mechanical stimulus rather than to a chemical one? The mechanical stimulus would appear to be in the nature of a change in tension or pressure, which stimulates growth just where the tension would be greatest, *i. e.*, at the borders of the depression. Küster carefully points out that such forces, besides others, are at play in callus formations. Cornu (9), who has made a very careful study of the root gall caused by this plant louse, and whose work has been either overlooked or disregarded by cecidiologists, likewise concludes that the sucking of the insect on the root, resulting in cessation of growth of the attacked part, produces tensions which dilate other elements not hindered in their growth.

In the description of the histological development of the gall when it is three to four days old, we noted that the upper half of the leaf directly below the insect showed no proliferation, while the under half, on both sides of the narrow portion, proliferated very abundantly. The lack of growth of the upper half of the leaf, which is directly below the insect, is not a specific character of this gall only but, as Cosens (10) points out, this phenomenon is the usual occurrence in the simpler

galls, in which the stimulus is applied in one direction only, and also exists in the highly complex Neuroterus gall described by Weidel (31). This latter author believes that mechanical stimuli are the factors in the production of even the most highly developed Hymenopterous galls. He questions Beyerinck's (1) hypothesis of chemical stimulation and asks why is it that the proliferation is more pronounced around the larva, *i. e.*, in tissues some distance away, than in tissues immediately in contact with it? Cosens, who believes that gall producers secrete enzymes which bring about cecidial formation, answers this question by saying that it seems likely that the enzyme content requires a certain degree of concentration in order to exhibit its maximum activity, and that immediately in contact with the larvae the enzymes do not possess the requisite degree of dilution to cause the greatest stimulation.

In the Phylloxera galls, as noted, the tissue next to the nymph shows no increase in the number of cells, and furthermore the tissue immediately around the proboscis showed no proliferation. If this insect introduces any diastatic enzymes, as Cosens believes, they must be introduced as salivary secretions by means of the proboscis, since secretions from such structures as cenocytes, as Rössig (25) describes, or Malphigian tubules, as Triggerson (28) describes, of the insect body, would be made in this case on exposed leaf surfaces with very little chance of entering the leaf. I have sprayed solutions of diastases on young vine leaves and observed no indication that it entered the leaf. According to Cosens's hypothesis the area immediately around the proboscis would not grow much, because the concentration of the enzymes would be too great, but further away, where the concentration of the enzymes would be less, growth would be greater. It is difficult on this basis to explain the phenomena under consideration. Cosens assumes that these enzymes are readily diffusible, an assumption which is not supported by investigation. Magnus (18) says that material coming from the insect which may play a part in gall formation does not have to be readily diffusible, but Küster (17) says: "Das einzige, was wir von den Eigenschaften der von den Cecidozoen gelieferten Stoffe wissen, ist, dass sie wasserlöslich sind und auf dem Wege der Diffusion durch Zahlreiche Zellschichten im Körper der Wirtspflanze sich verbreiten können."

Cosens performed several experiments, in which he placed the larvae of *Amphibolips confluens* on starch solutions and after a time

obtained a test for sugar. From this he concludes that the Cynipid larvae excrete an enzyme capable of changing starch to sugar. He states that at present there is a tendency to ascribe the stimulating agent in gall production to enzymatic action and he holds that this is a safe working hypothesis. He is careful to point out that only in the Cynipidae, referring to his own diastatic experiments, do we have experimental evidence for this. Magnus (18) says, not only the larvae of the Cynipidae give off diastatic and proteolytic enzymes, but larvae of the Diptera do the same thing. He describes an experiment in which larvae of *Dasyneura* (*Perrisia*) *terminalis* were placed on a starch-gelatin medium and remained alive for a long time. After twenty-four hours, the starch around them had been dissolved in a ring, showing a diastatic action, and similarly the gelatin had been dissolved by proteolytic enzymes. However, he does not conclude from this experiment that gall production may be traced to these enzymes, and very critically he says: "Erscheint also auch immerhin die Mitwirkung der vom Gallentier ausgeschiedenen proteolytischen Enzyme bei der Gallenbildung möglich, ist hierfür bisher kein Beweis erbracht, vielmehr gaben alle Versuche, mit proteolytischen Enzymen die Pflanzengewebe in andere Entwicklungsbahnen zu lenken, negative Resultate."

Performing experiments along the lines described above, I extracted hundreds of Phylloxera nymphs from galls and placed them on a starch solution. I obtained sugar tests in a number of such experiments, while checks gave no tests. I am not sure, however, that I did not introduce wild yeasts or other micro-organisms along with the nymphs. However that may be, when I sprayed young vine leaves with a fine spray of a watery solution in which the nymphs had been deposited, my results were negative. Injections of this solution into the leaves with fine glass tubes all gave negative results. Since only a small number of injections were tried, the results may not be conclusive.

Magnus (18), who has given us an excellent summary of the theories concerning the etiology of gall formation, concludes that the stimuli are not enzymatic as Beyerinck, Cosens, Molliard and others believe, or specific poisons, chemomorphs, as Küster and others maintain, but substances which inhibit the action of enzymes, substances which may be likened to the anti-enzymes of Czapek, antibodies of serum biologists, or hormones as described by Armstrong.

These substances, Magnus believes, are given off by the insect, or they may be the results of a material exchange between the living cells of parasite and host. They may produce osmotic disturbances, which will affect the nutritive processes of the plant tissue involved, and so give rise to gall production. This is quite hypothetical and as Magnus himself says, no direct evidence has been brought forth in support of the theory.

It is beyond the scope of this paper to go into all the theories put forth to account for gall production. Küster, in his articles and books, and Magnus (18) give thorough, up-to-date accounts of these discussions. Most of these are centered around a "chemical" theory, in which it is supposed that the producer injects some kind of chemical which serves as the stimulus for gall production. These theories are invoked to account for galls produced not only by one special class or order of insect gall producers, but for all gall producers, nematodes, mites, insects, and fungi. Küster, who attempts to classify cecidia on a structural basis, is an exception. He (16) thinks that different kind of stimuli may produce "organoid" galls from those which produce "histoid" galls. He is a firm believer in the "chemical" theory to account, at least, for his "histoid" galls. He pushes this theory a step further and says that certain kinds of chemicals, "chemomorphs," produce certain kinds of galls.

If any such chemical substances are injected into the vine leaf by the Phylloxera insect, it seems to me that their effect would be almost negligible as compared with the effect of a continuous sucking action for fifteen days at one fixed point, as far as *initial* stimuli produced by the insects are concerned. Here it should be pointed out that I have interested myself in the *initial* stimulus only. Undoubtedly the final stimuli for growth, in galls as well as for ordinary normal growth, are chemical stimuli; but, as Küster says, between the initial cause and the final effect there probably intervenes a "chain of stimuli." Thus wounding may release certain chemical stimuli which will bring forth callus formation [see Haberlandt (14)], but the initial stimulus is the wound.

Cornu (8 and 9), whose thorough, painstaking study of the root gall of *Phylloxera vastatrix* strikes one as being authoritative, concludes that the insect does not inject any poisons or other chemicals which are the stimuli for gall production. He gives the following reasons for this conclusion. First: the attacked rootlets first are made to take

the form of hooked swellings and then they die. If a poison is injected by the insect which produces first a swelling, why, he asks, does the same substance later on stop growth? Second: when roots of the vine attain a diameter of more than 3 or 4 mm., no swellings are produced, although a considerable number of Phylloxera are often seen on such roots. They are grouped or aligned in the bark cracks, alongside of each other, and if they give off an acrid, irritating fluid, they ought, united in a mass, to produce considerable disturbance and a proliferation of the elements of the cortical tissue. Their effect, however, is very feeble. Cornu says if the argument against this is offered, that the bark cells cannot respond because they are older cells, it may be pointed out that each year the old bark is exfoliated by means of a new suberized layer, coming from the embryonic tissue, the cork cambium. The effect produced is a hypertrophy and a coloring of certain gum reservoirs. Third: the galls of the stem and tendril are produced by a portion of the cortical tissue around the Phylloxera and not immediately below it. He says, it is not a local excess of acrid liquid concentrated at one point, which stops the formation of new tissues; for, in the cells more or less distant from this point, where the effect of this excess should be less effective, the hypertrophy should still manifest itself. This it does not do. Directly below the insect beneath a few layers of cells, is the generative zone, as Cornu points out, referring to the cambium layer of the stem or tendril. This zone does not produce any new growths. The swelling of the root is not produced under the insect, at the point punctured by it and where it produces a depression, but in the region farthest away, which makes up the hook form of the swellings. Fourth: if there has been a chemical irritant introduced, it should manifest itself by a swelling immediately in contact with the insect, for even after an attack of only several hours, there is found, around the point which has been occupied, an obvious depression. Cornu came to the conclusion that the puncturing by the proboscis together with the absorption of cell contents is sufficient to explain cellular segmentation and production of new tissue. To prove this contention and to show that irritating liquid had nothing to do with gall formation, Cornu injected into roots, stems, and leaves, 25 per cent. solution of acetic acid and 10 per cent. solution of sulphuric acid. Although in one or two cases he obtained swellings, he got no depressions, so that he is satisfied that

gall production is the result primarily of the sucking by the insect. His experiments on this point are very scanty and some of his reasons against chemical stimulation are open to question. Cornu noted that the sucking and puncturing stops the development of certain cells and as a result, he says, tensions are set up which cause neighboring cells to become dilated up to a certain size and then to divide. On the leaf the tensions occur on the underside, so the growth is in that direction, while on the rootlets the tension is felt on the sides, so the growth is at the sides of the insect.

Cook (5) studied the mouth parts of several Hemiptera gall producers, among which was *Phylloxera vastatrix*. He says: "So far as I have been able to determine, the insects do not remain attached to any one point for a great length of time." From this he concludes that the modification of plant tissue to form the gall is purely mechanical, being a continuous effort on the part of the plant to heal the wound produced by the repeated puncturing of the cells by the insect. Cornu (8) however, finds that the insect in the *Phylloxera vastatrix* leaf and root gall remains immovable, and fixed by its proboscis to the bottom of the cavity. My observations substantiate those of Cornu in that the insect, as soon as it fixes itself on the upper epidermis, seems to remain fixed for at least a considerable period. A proof for this is the frequency with which I have found the proboscis in the tissues in many of my prepared slides. But the immobility of the insect manifests itself in several other ways. Text-figure 2 shows how closely the insect fits into the cavity, so close in fact, that it could hardly move around in it, and in this case the insect has remained attached to the leaf from twenty-four to forty-eight hours. Again, cross sections of young and old galls show the proboscis usually at one fixed place, which place may be marked by a greater depth of the insect cavity, and by the broken-up epidermal and mesophyll cells, which are very few and are only found where the proboscis has penetrated. We may therefore conclude that Cook's hypothesis of a mechanical stimulus playing the part in gall production is not founded on observed facts, since the insect remains fixed and does very little puncturing.

The following facts stand out markedly in the formation of the Phylloxera gall: The young nymph attaches itself to the upper surface of a bud leaf. Within twenty-four hours, or less, the attacked portion shows a depression, the edge of which is bordered by hairs,

while the growth of the tissue in which the proboscis is working is arrested. Within thirty-six hours, the lower half of the leaf tissue, which is situated some distance from the portion attacked, begins to enlarge rapidly, giving rise to enormous hyperplastic growths. While the lower side of the attacked leaf has been growing enormously, the upper portion of the leaf directly beneath the insect, which shows at first an elongation of epidermal and palisade cells, does not increase in the number of cells. This portion becomes the bottom of the cavity, while the upper portion of the leaf, which surrounds the insect, greatly proliferates, grows upward and becomes the sides of the cavity. As the leaf grows, the growth of the gall proceeds, and at the end of twelve to fifteen days when the leaf is almost fully expanded, the gall has attained its full size and encloses the cecidial producer, which has increased in volume many times. The gall also contains several hundred eggs which the insect has laid. Text-figure 5 shows such a gall, partially cut open. The one thing that is definitely known concerning the work of the insect is that it has sucked for fifteen days at one very small area, and that it has obtained enough nutriment to feed itself and to enable it to produce several hundred eggs. This sucking action suggests itself as the initial stimulus in gall production. It appears to the writer that the disturbances which such an action would produce, such as the lowering of tensions at one part and the increase in another, the change in osmotic relationships necessary to counterbalance the withdrawal of cell contents, are factors which are sufficient to account for the localized abnormal growth.

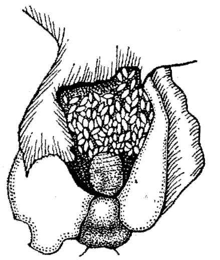


FIG. 5. An opened gall. Showing the gall producer and her eggs filling the cavity. (Note the narrowness of the gall where the insect is sucking.) Magnified 5 times.

Von Schrenk (30) produced intumescences on cauliflower leaves by spraying them with various copper salts. I have likewise produced these intumescences both on cabbage and on cauliflower. Figure 10 shows a cross section of a cabbage leaf with a large intumescence projecting from the lower side of the leaf. The leaf was sprayed with a very fine spray, only on the under side, with a solution of ammonium copper carbonate made up of 4.5 cc. ammonia, 0.5 g. copper carbonate and 750 cc. of distilled water. These hyperplastic

growths may be made to appear on the upper or lower surface of the leaf, depending on whether the leaf is sprayed on the upper or lower side.

In no case are depressions or cavities produced at the point of contact between the leaf tissue and the chemical applied. The growth response is always in the mesophyll tissue immediately below the point where the spray was applied and not on the side furthest away from the application of the spray. Sprays with commercial diastases on young cauliflower leaves likewise produced intumescences, but I feel that this latter experiment was not done on a large enough scale to warrant any conclusions. It is possible that other substances in the commercial diastase were factors in the production of the intumescences. If any conclusion may be drawn from these experiments it is that excessive growth takes place in those cells in which the applied chemicals are at their greatest concentration, and not at a distance from the center of application, where the concentration would be less.

If we compare these artificially produced intumescences with the Phylloxera gall which has been described above, it will be seen that in the intumescences produced by the application of chemicals, the place of application is the place of excessive growth, and in the Phylloxera the place of application is the place of hindrance of growth. From these experiments the burden of proof becomes more difficult for those who adopt the "chemical" theory of gall production for sucking insects. This is especially true in the case of the Phylloxera gall.

VIII. Summary

1. The *Phylloxera vastatrix* leaf gall starts to develop on embryonic bud leaves. In twenty-four hours the insect produces a depression at the periphery of which hairs are formed on the upper surface of the leaf. The depression is due to a lessened growth of the attacked mesophyll.

2. After three to four days of insect attack the lower half of the leaf tissue which surrounds the portion in which the proboscis is inserted has proliferated enormously. The whole thickness of the leaf in the region immediately around the proboscis shows no proliferation. That portion of the leaf which is beneath the insect does not proliferate, but the upper half at the sides of the insect grows upwards and forms the walls of a large insect cavity. Upper epidermal cells and several

layers of mesophyll cells in the portion of the gall below the insect, show peculiar thickening and dissolution of their cell walls.

3. Gall development depends upon leaf development; when the leaf reaches its maximum size, after twelve to fifteen days of development, the gall becomes mature.

4. A mature gall shows but slight cuticular development and very few stomata. The mesophyll is a huge mass of compact, thin-walled, partly empty cells, some of which are undersized, and others enormously elongated, the vascular elements are scattered by wedges of parenchyma cells. Many unicellular and multicellular hairs grow out from the gall.

5. Chemical work on this gall shows it to be a structure in which anabolic processes are lacking, and in which large amounts of simple sugars and simple proteins are present.

6. The development of this gall does not seem to support the theory that the insect injects some chemical into the leaf which causes gall formation.

7. Intumescences produced by chemical sprays result from entirely different kinds of hyperplastic responses than hyperplastic gall growth.

8. The investigation establishes the fact that the proboscis may pass through the entire thickness of the leaf.

9. The insect remains fixed, and that portion of the leaf in which the proboscis is fixed is marked by lack of growth as compared with the huge outgrowths which surround it.

10. The continuous sucking action by the insect at one fixed point for fifteen days is believed to be the initial stimulus for gall development.

The work on this paper was done in the Botanical Laboratory of the University of Wisconsin. My appreciation is due to Professor J. B. Overton, under whose direction the work was done, and to Professor W. S. Marshall for helpful suggestions and for the use of his private library.

U. S. National Museum

BIBLIOGRAPHY

1. **Beyerinck, M. W.** Ueber die ersten Entwicklungsstadien einiger Cynipidengallen. Verh. Nederl. Akad. Wetensch. 1882. (Amsterdam.)
2. **Barber, M. A.** A Technique for the Inoculation of Bacteria and Other Substances into Living Cells. Journ. Infect. Dis. **8**: 348. 1911.

3. —— The Pipette Method in the Isloation of Single Micro-organisms. Philippine Jour. Sci. 9. Sec. B., no. 4. 1914.
4. **Cook, M. T.** Galls and Insects Producing Them. Ohio Nat. **2**: 263. 1902.
5. —— Galls and Insects Prodncing Them. Ohio Nat. **4**: 124. 1904.
6. —— Insect Galls of Indiana. Ann. Rept. Dept. Geol. & Nat. Res. Ind. **29**. 1905.
7. —— The Insect Galls of Michigan. Mich. Geol. & Biol. Surv. Pub. 1, Biol. Ser. 1. 1910.
8. **Cornu, M.** Etudes sur la nouvelle maladie de la vigne. Mém. Prés. Div. Sav. Acad. Sci. Inst. Nat. France. **12**: No. 6. 1876.
9. —— Etudes sur le Phylloxera vastatrix. Mém. Prés. Div. Sav. Acad. Sci. Inst. Nat. France. **26**. 1878.
10. **Cosens, A.** Contribution to the Morphology and Biology of Insect Galls. Trans. Canad. Inst. **9**: 297. 1912.
11. **Czapek, F.** Stoffwechselprozesse in der geotropisch gereizten Wurzelspitze und in phototropisch sensiblen Organan. Ber. Deutsch. Bot. Ges. **20**. 1902.
12. **Dorrance, Frances.** Küster's Pathological Plant Anatomy. 1913.
13. **Haberlandt, G. F.** Ueber experimentelle Hervorrufung eines neuen Organs bei Conocephalus ovatus. Trec. Festchr. Schwendener. **104**. 1899.
14. —— Zur Physiologie der Zellteilung. Sitzungsber. Preuss. Akad. 318. 1913.
15. **Houard, C.** Les zoocecidies des plantes d'Europe et du Bassin de la Mediterranée. 1908. (Paris.)
16. **Küster, E.** Die Gallen der Pflanzen. 1911. (Leipsig.)
17. —— Gallen. Handwörterbuch der Naturwissenschaften **4**: 1913.
18. **Magnus, W.** Die Entstehung der Pflanzengallen verursacht durch Hymenopteren. 1914. (Jena.)
19. **Magnus, P.** Ueber eine neue unterirdisch lebende Art der Gattung Urophlyctis. Ber. Deutsch. Bot. Ges. **19**: 145. 1901.
20. **Molliard, M.** Recherches physiologiques sur les galles. Rev. Gen. Bot. **25**. 1913.
21. —— Effets de la compression sur la structure des racines. Rev. Gen. Bot. **25** bis: 529. 1914.
22. **Pantanelli, E.** Ricerche fisiologiche su le viti Americane oppresse da galle Fillosseriche. Staz. Sperim. Agr. Ital. **42**: 305. 1909.
23. **Râthay, E.** Das Auftreten der Gallenlaus im Versuchsweingarten zu Klosterneuberg im Jahre 1887. Verhandl. Zool.-Bot. Ges. Wien. **39**: 47. 1889.
24. **Riley, C. V.** The Grape Phylloxera. 6th and 7th Ann. Rpts. Noxious, Beneficial and other Insects of the State of Mo. 1874 and 1875.
25. **Rössig, H.** Von welchen Organen der Gallwespenlarven geht der Reiz zur Bildung der Pflanzengalle aus? Zool. Jahr. System. **20**: 19. 1904.
26. **Sorauer, P.** Handbuch der Pflanzenkrankheiten. **1**: 296. 1886.
27. **Stebbins, Fannie A.** Insect Galls of Springfield, Mass. and Vicinity. Springfield Mus. Nat. Hist. Bull. **2**: 1910.
28. **Triggerson, C. J.** A Study of Dryophanta erinacii and Its Gall. Amer. Ent. Soc. **7**. No. 1. 1914.
29. **Viala, P.** Les maladies de la vigne. 1893. (Paris.)

30. **Von Schrenk, H. R.** Intumescences as a Result of Chemical Stimulation. Rep. Mo. Bot. Gard. **1905**, 125.
31. **Weidel, F.** Beiträge zur Entwicklungsgeschichte und vergleichenden Anatomie der Cynipidengallen der Eiche. Flora **102**: 279. 1911.

DESCRIPTION OF PLATES XIV AND XV

All the figures are microphotographs taken with various Leitz and Zeiss objectives and eyepieces.

FIG. 1. A cross section of an embryonic bud leaf 24 hours after insect attack showing the formation of hairs on the upper and lower leaf surfaces. On the upper surface the hairs are produced only at the sides of the insect. Magnified 102 ×.

FIG. 2. A longitudinal section of a primary vein of a bud leaf. The insect is partially withdrawn from her normal position, but the end of her proboscis is still projected into the upper part of the vein. The beginning of hair formation on the upper surface of leaf and vein may be seen on both sides of the insect. Magnified 127.5 ×.

FIG. 3. A cross section through the center of a three to four day old gall. Showing proboscis protruding through the entire width of the leaf, and showing the narrowness of the gall where the proboscis is working. Magnified 119 ×.

FIG. 4. A higher magnification of the central part of Fig. 3. Magnified 212.5 ×.

FIG. 5. A cross section of a mature gall showing among other things bent parenchyma cells. Magnified 14.5 ×.

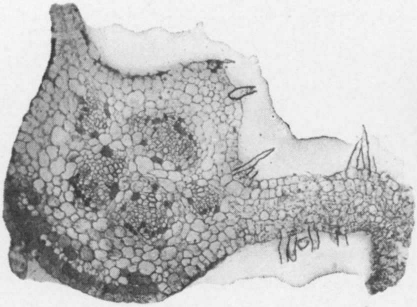
FIG. 6. A cross section near the center of a mature gall showing the mouth of the gall, the scattering of the vascular elements, etc.

FIG. 7. A cross section of three layers of cells immediately below the insect in a three to four day old gall. The thickening and dissolution of the cell walls, giving the appearance of a reticulate structure of tracheae is shown. Magnified 498 ×.

FIG. 8. A cross section through a mature gall showing the nutritive zone, nymphs and eggs in the cavity, etc. Magnified 14 ×.

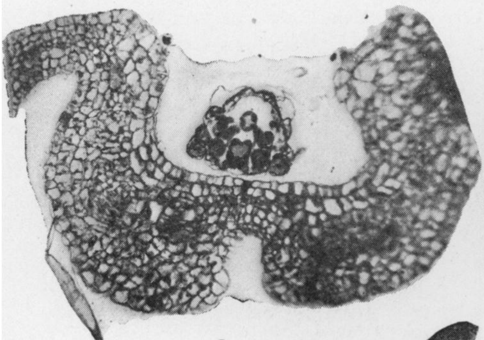
FIG. 9. A cross section of an embryonic bud leaf, showing the first signs of gall formation, the proboscis protruding from the upper epidermis, the narrowness of the leaf at this point and the beginning of hair formation at the sides of the insect. Magnified 127 ×.

FIG. 10. A cross section of an intumescence, produced on a cabbage leaf by a spray of ammonium copper carbonate. Magnified 53 ×.



1

2

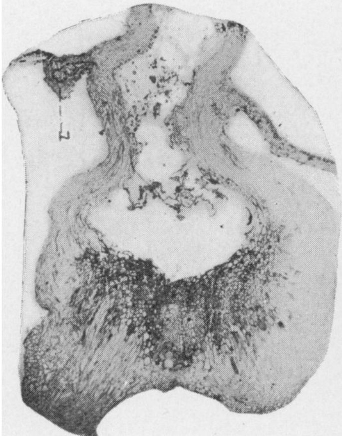


3

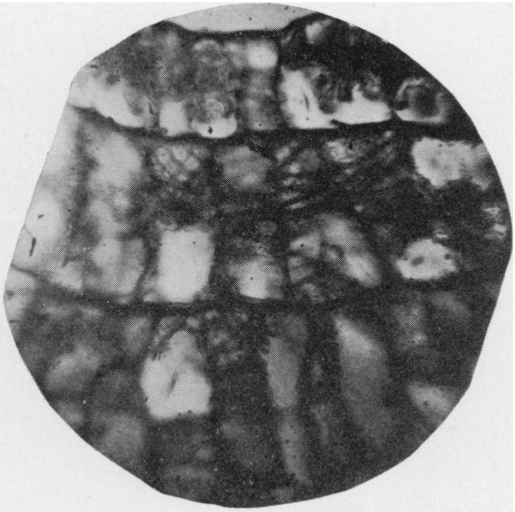
4

5

ROSEN: PHYLLOXERA LEAF GALL.

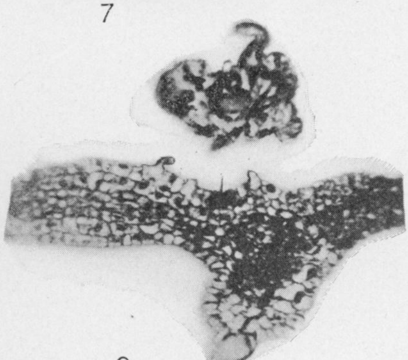


6

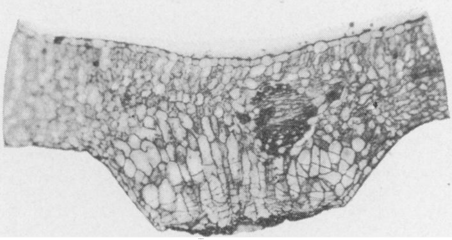


7

8



9



10

ROSEN: PHYLLOXERA LEAF GALL.